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Supplementary Information

Label-Free Electrical Assay of Fibrous Amyloid β Based on Semiconductor Biosensing

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1. Materials

The amyloid proteins, Aβ42 and Aβ40, were purchased from Peptide Institute, Inc. and Congo red (CR) from Tokyo Chemical Industry Co., Ltd. The self-assembled monolayer reagent, 3-aminopropyltriethoxysilane (APTES), and human serum albumin (HSA) were purchased from Sigma–Aldrich Inc. The semiconductor-based field effect transistor (FET) biosensor was obtained from Toppan Printing Co., Ltd. The other chemicals were purchased from Kanto Chemical Co. Inc. The buffer, phosphate buffered saline (1× PBS) of pH 7.4, was made by using 137 mM NaCl, 8.1 mM $Na_2HPO_4•12H_2O$, 2.7 mM KCl, and 1.5 mM KH_2PO_4. Diluted PBS, 0.01 × PBS (pH 7.4), was prepared by diluting 1× PBS with ultrapure water.

2. Preparation of amyloid beta (1-42) isoform

The amyloid β peptides, Aβ42 and Aβ40, were dissolved in 0.1% ammonia water, followed by the dilution with PBS, to obtain 100 μM concentration. Subsequently, the solution was incubated for 0-3 days at 37°C in a 1.5 mL tube. Additionally, the sample of Aβ42 was diluted with PBS to a required concentration in the range of 100 fM to 100 μM.

3. Functionalization of semiconductor FET-based device for amyloid β detection

The surface of silicon dioxide as a gate insulating film of the semiconductor-based FET biosensor was exposed to O_2 plasma (200 W for 1 min) to introduce hydroxyl groups on the surface, followed by coating with SAM of APTES. First, the SAM was formed on the silicon dioxide surface by immersing in 1% (v/v) APTES in toluene at 60ºC for 7 min in an argon atmosphere. After the SAM modification, for cross-linker,

glutaraldehyde (GA) was allowed to react with the amino-terminated surface by immersing a gate area of the aminopropylsilane (APS)-modified FET in a solution of 2.5% GA in 1× PBS for 30 min. Subsequently, the probe molecule CR was allowed to react with the aldehyde moiety of GA-modified surface for 60 min. This reaction resulted in the fabrication of the CR-immobilized FET. The gate voltage (V_g) - drain current (I_d) relation of the CR-immobilized FET was measured and used as the reference. The measurements were made in the dark with a semiconductor parameter analyzer (2612A, Keithley Instruments Inc., USA) at room temperature in 0.01× phosphate buffered saline (PBS, pH 7.4) by sweeping the V_g from -3 V to 1 V with a 0.1 V drain voltage. The reference electrode was Hg/Hg_2SO_4. The CR-immobilized FETs were immersed in the Aβ solutions (Aβ42 and Aβ40), which were prepared by following the above-mentioned procedure, for 30 min. After the immersion, the residue was washed with 1× PBS, followed by 0.01× PBS. The V_g - I_d characteristic of the Aβ-reacted FET was measured in 0.01× PBS and compared with the reference. The threshold voltage shift (ΔV_g) was calculated.

4. Optimization and characterization of the CR-immobilized surface

The state of the immobilization of CR molecule on the surface of SiO_2 substrate was characterized by XPS. XPS measurements were performed on a spectrophotometer (PHI-5000 Versa Probe WS, ULVAC-PHI Inc.) using an Al Kα X-ray source. The three specimens were examined: the specimens treated with the CR solution of either 10 ng/mL or 1 μg/mL, hereafter denoted as “10 ng/mL CR” and “1 μg/mL CR”, respectively, and the specimen without CR treatment, denoted as “GA-modified”. As compared with the “GA-modified” specimen, the increase in the peak intensity was

observed for both C 1s and N 1s regions after the treatment with CR. Here the increment from "GA-modified" state for "1 µg/mL CR" was greater than that for "10 ng/mL CR". For the S 2p region, a weak but a certain component was observed at the binding energy between 167 and 169 eV for "1 µg/mL CR", which was not observed for "GA-modified". Here, a very weak S 2p component appeared at the same binding energy for "10 ng/mL CR". Considering the chemical structure of CR molecule, those are attributable to the immobilization of CR molecules on the surface.

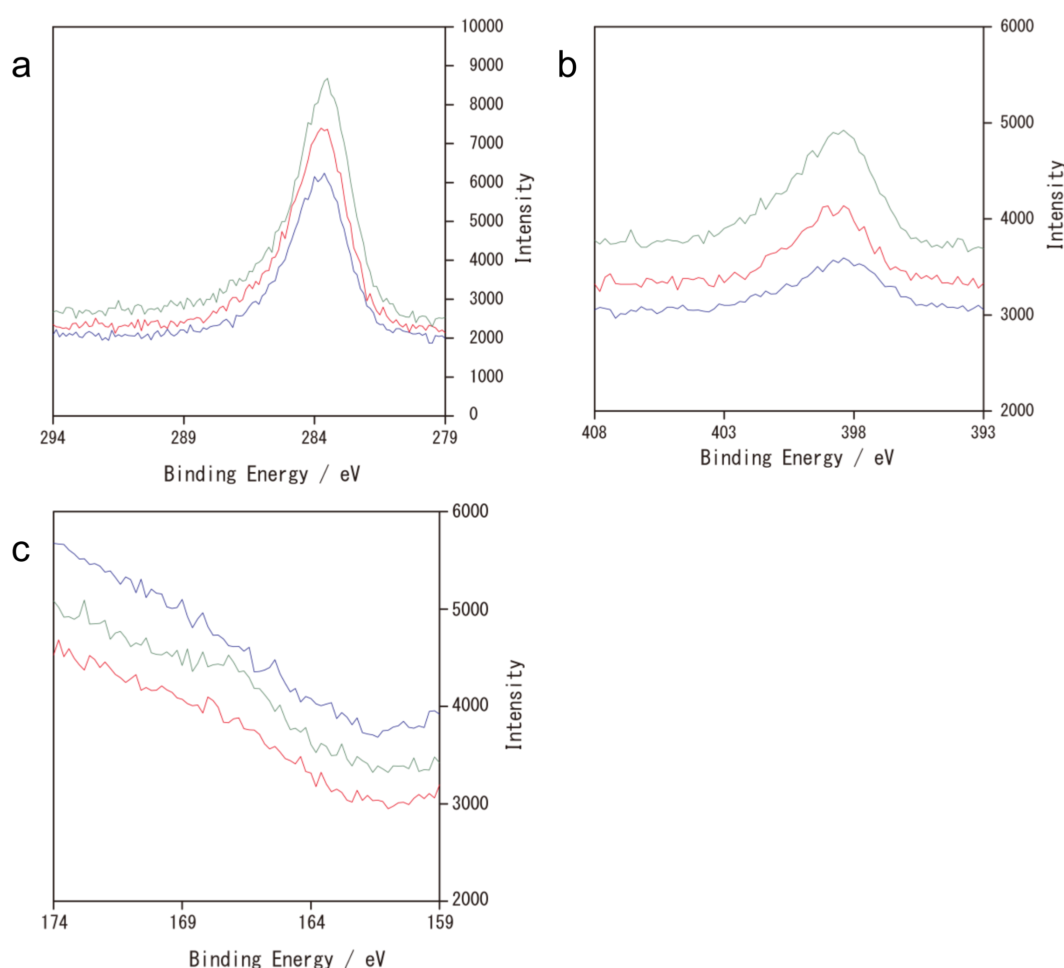

Figure S1. X-ray photoelectron spectra of (a) C 1s, (b) N 1s, and (c) S 2p regions of GA-modified SiO_2 specimens before (blue, "GA-modified") and after the treatment with CR solution (red, "10 ng/mL CR" and green, "1 µg/mL CR").

In our preliminary study, we confirmed that the concentration of congo red (CR) solution for immobilization have an effect on the degree of interaction between CR molecules and yeast prion Sup35NM.[1] After the experiments, 10 ng/mL CR was determined to be the optimal concentration for CR immobilization of the FET gate surface. The signals decrease as the CR concentration is increased by more than 10 ng/mL, which is assumed to be related to the formation of helical aggregates of CR molecules at higher concentrations through intermolecular π–π interactions in solution, like dimers or oligomers, impairing the binding specificity of the aggregated CR probe to the fibril proteins.

Reference

1. S. Hideshima, S. Wustoni, S. Kuroiwa, T. Nakanishi, A. Koike and T. Osaka, *ChemElectroChem*, 2014, **1**, 51-54.